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Radio frequency energy effects on microorganisms in foods ☆

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Abstract

Liquids containing microorganisms were exposed to radio frequency (RF) energy to study non-thermal inactivation. RF energy was applied to the liquids while heat was simultaneously removed to control temperature. Turbulent flow was maintained to minimize localized heating. An 18 MHz RF processor applied an approximately 0.5 kV/cm electric field strength to the liquids. It was capable of pasteurizing the liquids provided that cooling was minimized. There were no non-thermal effects of RF energy detected on *Escherichia coli* K-12, *Listeria innocua*, or yeast in apple cider, beer, deionized water, liquid whole egg, and tomato juice; nor were there any synergistic effects of RF energy with heat. The low temperature effects of RF energy at 18 MHz and 0.5 kV/cm were due to heat.

Keywords: Apple cider; Food safety; Liquid egg; Non-thermal; Pasteurization; Radio frequency

Industrial relevance: There has been a long and controversial discussion regarding the non-thermal effects of radio frequency (RF) energy on microorganisms. The benefits of pasteurizing liquid foods with low electric field strength and low power would be enormous. In this thorough study every effort has been made to ensure accurate temperature measurements to obtain more conclusive evidence relating nonthermal effects of RF. There was no evidence found that the given RF energy of 18 MHz and an electric field strength of approximately 0.5 kV/cm can inactivate microorganisms in liquids without heat. However, the authors indicate at 10kV/cm this might occur.

1. Introduction

For more than 50 years, the existence of a non-thermal effect of radio frequency (RF) energy on microorganisms has been debated. Fresh-like qualities and nutrients would be maintained in foods if RF energy could non-thermally inactivate microorganisms. For example, liquid foods such as whole eggs, fruit and vegetable juices, as well as beer, could be pasteurized with minimal degradation.

Decareau (1985) provided an excellent review of the early work on the effects of RF energy on microorganisms. Fleming (1944) concluded that, in the range of 11–350 MHz, inactivation of *Escherichia coli* was easy to obtain even with small amounts of power, e.g. 10 W. Brown and Morrison (1954), however, could not repeat Fleming's work.

Nyrop (1946) reported 99.5% kill of *E. coli* at ≤ 40 °C by applying a 0.23 kV/cm electric field strength at 20 MHz for a total of 7 s. However, details of the experimental apparatus and procedures were not described. Nyrop also claimed inactivation of the 'foot and mouth' virus by applying the same field strength for 10 s at ≤ 36 °C; whereas, inactivation using heat requires 60 h at 37 °C.

Ingram and Page (1953), aware of the work of Nyrop, studied the effects of 10 and 20 MHz radio frequency energy on *E. coli*, tobacco mosaic virus, baker's yeast, and bacteriophages. The samples were maintained below 30 °C and subjected to voltage gradients up to 2 kV/cm. No significant effects were observed under these conditions.

Carroll and Lopez (1969) exposed *Saccharomyces cerevisiae*, *E. coli*, and *Bacillus subtilis* to RF energy at 60 MHz at 49 °C and concluded that inactivation was

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due solely to heat. Furthermore, exposing enzymes to 60 MHz RF energy yielded the same conclusion (Lopez & Baganis, 1971).

More recently, Ponne, Balk, Hancioglu and Gorris (1996) studied the effect of RF energy on liposomes, yeast and bacteria. These materials were exposed to frequencies ranging from 1 to 100 MHz and an electric field strength of ≤ 0.34 V/m, while being maintained at ≤ 40 °C. Although exposure of liposomes to frequencies of 27 and 100 MHz resulted in increased lysis of vesicles, no effects were demonstrated when RF energy was applied to *S. cerevisiae* and *Erwinia carotovora*. This led Ponne et al. (1996) to conclude that there is no non-thermal effect of RF energy on microbial inactivation.

Numerous studies have been reported on non-thermal effects of 2450 MHz microwave energy on microorganisms in foods. Kozempel, Cook, Scullen and Annous (2000) summarized the theories that have been advanced to explain non-thermal effects of electromagnetic energy and investigated the effect of microwave energy on yeast and bacteria at ≤ 45 °C. They concluded that there were no non-thermal effects. Welt, Tong, Rossen and Lund (1994) concluded that the effect of microwave energy on the viability of *Clostridium sporogenes* spores was indistinguishable from the effect of conventional heating.

In summary, several studies have claimed that RF energy inactivates microorganisms by non-thermal means. However, there is no evidence that this technique is being practiced today by the food industry (Decareau, 1985; Mertens & Knorr, 1992). Some of the studies that disclaim the existence of non-thermal effects suggest that erroneous temperature measurements are the cause of the 'positive' results reported by others. In this study, every effort has been made to ensure accurate temperature measurements to obtain more conclusive evidence with regard to non-thermal effects. The apparatus described by (Brunkhorst, Ciotti, Fredd, Wilson, Geveke & Kozempel, 2000) was used to separate non-thermal from thermal effects.

The benefits of pasteurizing liquid foods with a low electric field strength, and low power, would be enormous. The purpose of this work was to establish the feasibility of non-thermal inactivation of microorganisms using RF energy.

2. Materials and methods

2.1. Equipment

Experiments were performed in which liquids were exposed to radio frequency (RF) energy while simultaneously being cooled. The equipment consisted of a double pipe heat exchanger inside a RF processor. The equipment has been extensively described (Brunkhorst et al., 2000). The outer pipe was Teflon, which is transparent to RF. The inner pipe was stainless steel and was grounded. Process fluid flowed up through the annulus and absorbed the RF energy. Turbulent flow ($Re = 6000$) within the treatment zone ensured temperature uniformity and eliminated local temperature gradients or hot spots. Water concurrently flowed in the inner pipe and removed heat from the process fluid. The upward flows minimized the amount of air in the double pipe.

A schematic of the experimental system is shown in Fig. 1. It includes a 190 l stainless steel feed tank. A sanitary positive displacement pump (Tri-Clover, Kenosha, WI, model PR3-1M-YH6-ST-S) supplied the feed to the RF processor at flow rates of 0.1 and 3.0

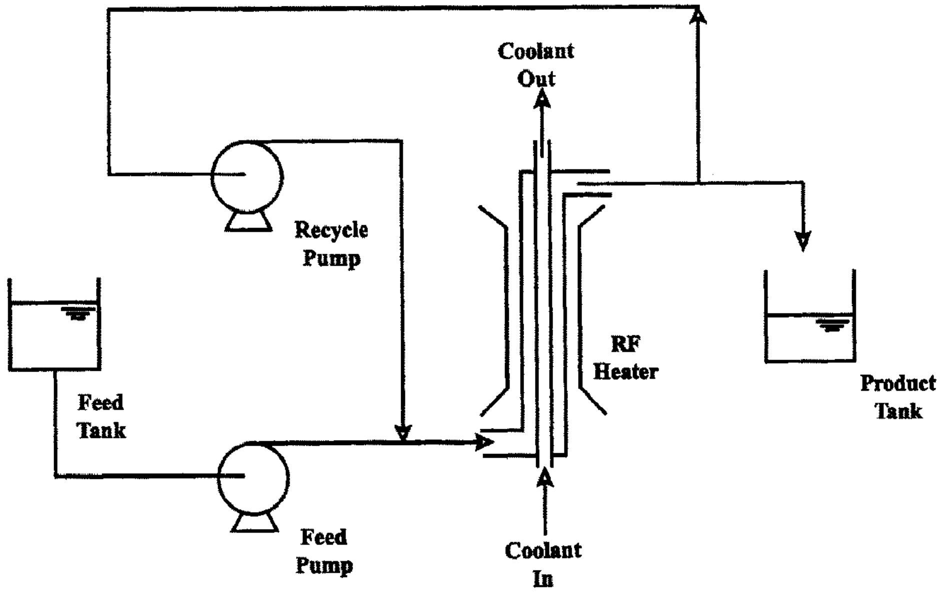

Fig. 1. Schematic process flow diagram of the RF system.

kg/min. The RF processor (PSC, Cleveland, OH, USA) supplied up to 19 kW of power at a frequency of 18 MHz and a field strength of 0.5 kV/cm. The outer pipe of the double pipe heat exchanger had an inner diameter of 3.8 cm. The inner pipe had an outer diameter of 2.5 cm. The length of the RF treatment zone was 86 cm. To increase the uniformity of RF treatment, a second sanitary positive displacement pump (Tri-Clover, model PR25-1$\frac{1}{2}$M-UH4-ST-S) recycled the process fluid. The recycle pump circulated the fluid at 9 kg/min, sufficient to achieve turbulent flow. Process fluid exited the recycle loop in direct proportion to the feed rate established by the feed pump.

Based on the dimension of the treatment zone, as well as the feed and recycle rates, the process fluid on average was exposed to either 110 million cycles (9 s) or 3.2 billion cycles (4.2 min) of RF energy.

The system can be visualized as a continuous stirred tank that receives RF energy. Liquid food, laden with microorganisms, enters the continuous stirred tank, is exposed to RF energy in the tank, and exits as a product stream with the same microorganism concentration as the tank. In a commercial unit, the recycle loop can be eliminated; however, it may be necessary to lengthen the RF zone or to use multiple zones to ensure uniform treatment.

The temperatures of the feed tank, water, and process fluid immediately before and after the RF treatment zone were measured with fiber optic sensors (Luxtron, Santa Clara, CA, USA, model 950). The temperatures were continuously logged to a data acquisition system (Dasytec USA, Amherst, NH, Dasylab version 5.0).

2.2. System preparation

The system was sanitized before introducing the test fluid by heating circulating water to over 65 °C using the RF processor with minimal cooling. Approximately 110 kg of feed was charged to the feed tank in each experiment after the process equipment was sanitized. Apple cider was purchased from a local producer. Beer was made from commercially available beer brewing kits. Tomato juice was purchased from a local distributor. Liquid whole egg was purchased from a local processor.

In some tests, the feed was inoculated with a test microorganism. *Escherichia coli* K-12 and *Listeria innocua* SA3-VT were supplied by P.M. Fratamico and L.K. Bagi, respectively, both of the US Department of Agriculture, Wyndmoor, PA, USA. The bacteria were maintained on tryptose agar (Difco Laboratories, Detroit, MI, USA) at 4 °C. The *L. innocua* and *E. coli* K-12 were grown in a brain–heart infusion (Difco Laboratories) for 24 h at 28 and 37 °C, respectively. The feed tank was inoculated from the culture to give a concentration of approximately 6 log cfu/ml.

After inoculation, the feed was pumped through the equipment for 10–15 min, displacing the sanitized water. When the system had been thoroughly flushed with the inoculated feed the RF processing began.

2.3. Sampling and analysis

Duplicate samples were taken periodically from the feed tank and product. Appropriate dilutions of the samples were plated on tryptose agar using a spiral plater (Spiral Biotech, Bethesda, MD, USA; model Autoplate 4000). Samples containing *E. coli* K-12 and *L. innocua* were incubated for 24 h at 28 and 37 °C, respectively. Samples containing microorganisms occurring in beer were incubated at 37 °C for 48 h. Enumerations were made with a colony counter (Spiral Biotech, model 500A).

3. Results and discussion

A test of the experimental equipment's ability to control product temperature was to run the process at normal pasteurization temperature, 60–65 °C, and, without changing any process variables such as power input or product flow rate, to drop the temperature to ≤ 45 °C using only cooling water in the inner pipe. For example, tap water with no added microorganisms was processed at normal pasteurization temperature, as shown in Fig. 2. Cooling water in the inner pipe quickly reduced the product temperature to 40 °C.

Having been assured that the system performed as intended, experiments with microorganisms in food were carried out. Apple cider was inoculated with *Escherichia coli* K12 to a microbial concentration of 5.5 log cfu/ml. After sanitizing with hot water, the system was flushed with the inoculated feed for 10 min. During this period, the concentration of *E. coli* in the product increased from < 1.3 log cfu/ml, the lowest detectable level, to that of the feed as shown in Fig. 3. At this point, the radio frequency (RF) processor was started and an electric field strength of 0.5 kV/cm was applied. The product temperature quickly increased and was controlled at 45 ± 1 °C using 24 °C cooling water. Samples were taken after steady state had been reached, based on the time calculated to flush the system three times. As shown in Fig. 3, the feed and product concentrations were equal, i.e. the concentrations were not significantly different (critical value of Student's t-test, $P > 0.05$). A non-thermal effect of RF energy was not found. Identical results were obtained for all of the liquids investigated in this study, as listed in Table 1.

A synergistic effect of RF energy and thermal energy would be valuable to the food industry because thermal exposure could be reduced. Experiments were per-

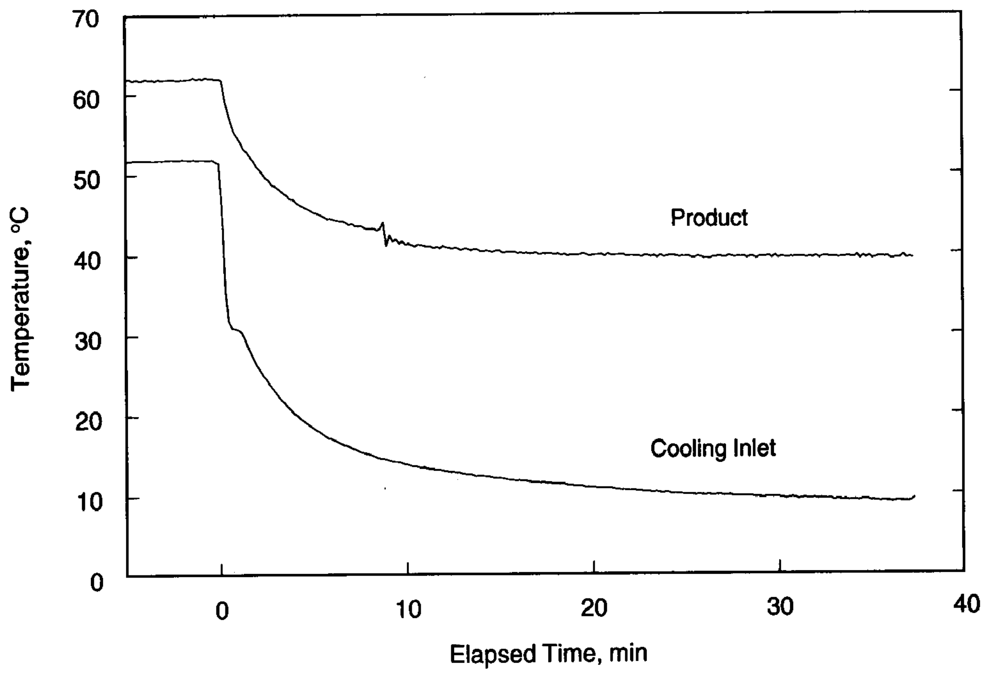

Fig. 2. Temperature control test. Process temperature controlled by only varying the cooling water inlet temperature. All other parameters kept constant: RF power supplied, 19 kW; feed rate, 3 kg/min; recycle rate, 9 kg/min.

formed near the inactivation temperatures of the test organisms to determine if a synergism exists. A control was performed in which the fluid was thermally processed without the RF processor on to differentiate between the effects of RF energy and other causes, such as heat. Hot water in the inner pipe heated the process fluid during this control.

Fig. 4 presents the results of an experiment in which apple cider was inoculated with *Listeria innocua* to 5.8 log cfu/ml. After sanitizing with hot water, the system was flushed with the inoculated feed for 15 min. During this period, the microbial concentration in the product increased from undetectable to that of the feed. The temperature of the water in the inner pipe was then

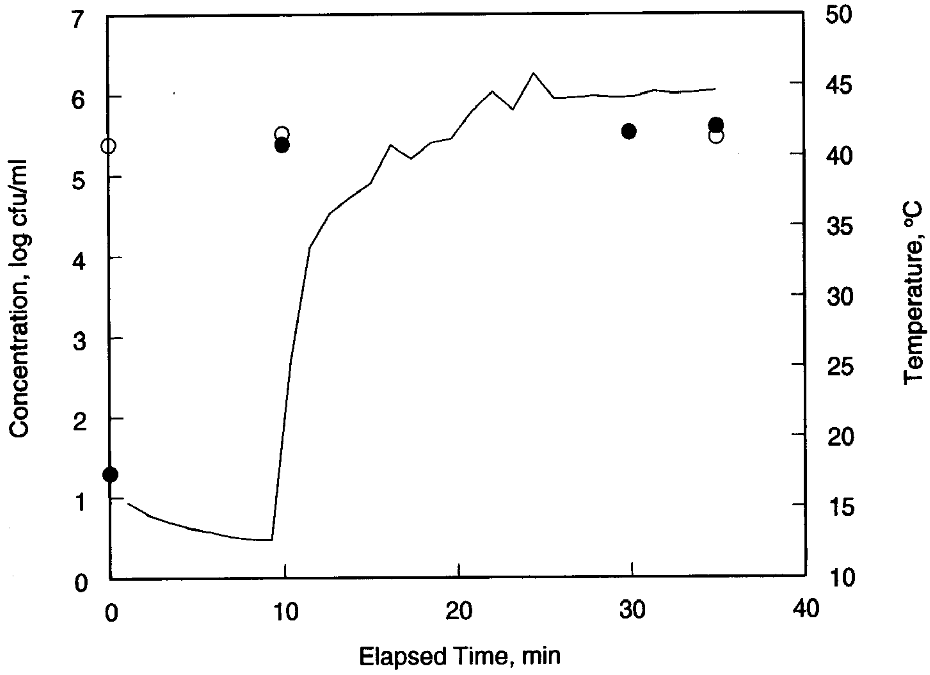

Fig. 3. Effect of RF energy on *E. coli* K12 in apple cider at 45 °C. After flushing the system with inoculated feed for 10 min, RF processing at 0.5 kV/cm was initiated. (— = Product outlet temperature, ○ = feed concentration, ● = product concentration.) Feed and recycle rates: 3 and 9 kg/min, respectively. Process fluid exposed to 110 million cycles of RF energy.

Table 1
Systems not exhibiting non-thermal effects

Material	Microorganism	Temperature, °C
Apple cider	*E. coli* K12	45
Apple cider	*L. innocua*	45
Beer	Naturally occurring	45
Deionized water	*E. coli* K12	35
Deionized water	*L. innocua*	45
Tomato juice	*L. innocua*	45
Whole liquid egg	*E. coli* K12	45
Whole liquid egg	*L. innocua*	45

raised to control the temperature of the apple cider at 50.5 ± 1 °C. After thermally processing for 50 min, the concentration of *L. innocua* had significantly decreased to 4.5 log cfu/ml ($P < 0.05$). Then, the RF processor was started and the temperature of the water in the inner pipe was reduced to approximately 36 °C to maintain the apple cider temperature at 50.5 ± 1 °C. After RF processing for 50 min with a field strength of 0.5 kV/cm, the concentration of *L. innocua* also had significantly decreased to 4.7 log cfu/ml ($P < 0.05$). However, the reduction in both cases was not significantly different ($P > 0.05$). Therefore, a synergistic effect of RF energy was not found. Similar results with identical conclusions were obtained for all of the liquids investigated in this study, as listed in Table 2.

The results of this study support the opinion that the sole effect of RF energy on the inactivation of microorganisms is due to heat (Decareau, 1985; Mertens & Knorr, 1992). A possible explanation for the lack of non-thermal effects seen in this study and others, previously cited, is that the electric field strength was not high enough. According to the dielectric rupture theory, an external electric field induces a transmembrane electric potential (Zimmermann, Pilwat & Riemann, 1974). At sufficient potential, the cell membrane ruptures. Sale and Hamilton (1967) applied square wave d.c. pulses to suspensions of vegetative bacteria and yeast cells. They, as well as others (Hulsheger, Potel & Niemann, 1981; Simpson, Whittington, Earnshaw & Russell, 1999), found that a minimum electric field strength of ≥ 5 kV/cm is required for inactivation. Kotnik, Miklavcic and Slivnik (1998) recently reported on the time course of transmembrane voltage induced by RF fields. Although they stated that it is generally very difficult to predict the peak value of the induced transmembrane voltage, it is clear from their analysis that, for a given field strength, the voltage is significantly reduced as the frequency is increased from 100 kHz to 1 MHz. Qin, Zhang, Barbosa-Canovas, Swanson and Pedrow (1994) exposed *E. coli* to oscillatory decay pulses with an undamped natural frequency of 100 kHz and to exponential decay pulses. At equal peak field strengths for both waveforms of 40 kV/cm, they observed that the exponential decay pulses resulted in more inactivation.

The applied field strength of the RF treatment chamber in the present study was limited to approximately 0.5 kV/cm by geometric and material constraints. At the frequency used, 18 MHz, the reduction

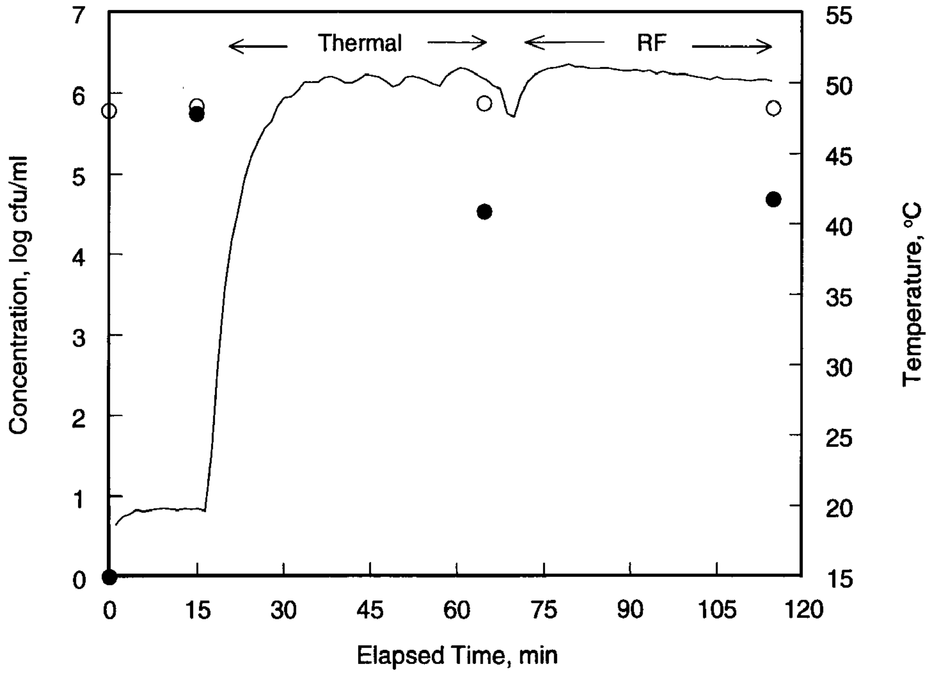

Fig. 4. Effect of RF energy on *Listeria innocua* in apple cider at 50.5 °C. After flushing the system with inoculated feed for 15 min, thermal processing was initiated. After thermally processing for 50 min, RF processing at 0.5 kV/cm commenced. (— = Product outlet temperature, ○ = feed concentration, ● = product concentration.) Feed and recycle rates: 0.1 and 9 kg/min, respectively. Process fluid exposed to 3.2 billion cycles of RF energy.

Table 2
Systems not exhibiting synergistic effects

Material	Microorganism	Temperature °C	Energy source	Concentration[a], log cfu/ml	
				Feed	Product
Apple cider	*L. innocua*	50.5	Thermal	5.9	4.5
Apple cider	*L. innocua*	50.5	RF	5.8	4.7
Beer	Naturally occurring	50.0	Thermal	6.3	4.4
Beer	Naturally occurring	50.0	RF	6.3	4.3
Deionized water	*L. innocua*	55.5	Thermal	5.9	4.4
Deionized water	*L. innocua*	55.5	RF	5.9	4.3

[a]S.D. for all concentrations were ≤ 0.1 log cfu/ml.

in induced transmembrane voltage would be significant. Therefore, to possibly succeed at non-thermally inactivating microorganisms by RF energy, the applied electric field strength must be increased and/or the frequency must be lowered. The field strength should be increased by one or two orders of magnitude. A new RF treatment chamber is being developed in order to study this. An alternative solution may be to use a lower frequency RF processor.

4. Conclusions

There was no evidence that RF energy at 18 MHz and an electric field strength of approximately 0.5 kV/cm can inactivate microorganisms in liquids without heat. Furthermore, there was no evidence to support the view that there is a synergistic effect of RF energy and thermal energy.

It may be possible, however, to non-thermally inactivate microorganisms at lower frequencies and/or higher electric field strengths with a new RF treatment chamber applying a field strength of 10 kV/cm.

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